Acta Biologica Szeged. *22* (1—4), pp. 57—71 (1976)

A. BIOLOGICAL ACTIVITY, BACTERICIDAL EFFECT AND STRUCTURE OF A MEDICINAL PEAT-BOG MUD (AT HÉVÍZ)

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(Received September 18, 1975)

Abstract

A study was made fo the curative effects of a peat-bog mud (at Hévíz, Hungary). It was found that, during the treatment of patients, the mud becomes richer in total nitrogen and mainly binds protein compounds. It was shown that the medicinal mud contains appreciable biologically-active substances, and also exhibits a bactericidal effect. The solid structure of the mud is a network of plant tissue remains, covered with humates, which possesses a high adsorption-capacity. The nitrogen- and carbon-, as well as organic-matter content of the medicinal mud of peat-bog in the lake at Hévíz and its drainage channel considerably differs from (is smaller than) that of the peaty soil of the environment, not covered by thermal waters (the C:N ratio is above 30). This particular combination, poor mainly in nitrogen, is due to the eluating effect of the rushing up and ceaselessly streaming thermal water. In a case like this, N is completely bound, immobile, cannot be drawn even by bacteria. The bacterial content of mud is, therefore, very small.

The total N-content of the muds already used to packing therapy is 27 per cent higher and its soluble total protein concentration grew seven to one. By thermal mud, therefore, some N compound of considerable quantity is eluated from the surface tissues of man and bound. This phenomenon may be regarded as one of the cause of the curative effect of mud.

The growth of roots of the germinating seeds was stimulated 100 to 500 per cent by the aqueous extract of mud. The thermal mud therefore contains considerable biologically active substances that, getting in through the surfaces treated, may be the second main cause of the curative effect.

It was established by the aerobic method of agar-diffusion that a third curative effect of our thermal mud is its bactericidal effect.

By means of microscopic investigations the solid, organic frame of mud turned out to consist of reticular vegetable structures of large surface covered by a mantle of dark humic acid. The remarkable adsorptive capacity of the mud at Hévíz that may similarly be classified into its mechanisms of curative effect is guaranteed by the humic acids and this reticular-spongious structure.

Introduction

The lake bath at Hévíz and the indications of mud therapy became Known most widely by the chronic locomotor diseases and the rheumatological treatments respectively.

The peaty medicinal mud at Hévíz, as established by BREZNAY (1970), is a natural mixture of various kinds of mud. The author describes in detail the origin, classification, physical and chemical state of mud.

As regards balneotherapy, it was established by STRECKER (1970) that — in contradistinction to the exact medicine — it was considerably founded upon empirism and complexes to be analysed but with difficulty. In his opinion, the good "cureeffect" as described in the final reports is generally accepted by clinicians only with reservations because the question arises: "What was effective?"

In literature, radioactivity, sulphuric content, an other inorganic cations and anions have primarily a part as the effective agents of thermal water and, partly, of mud too.

But the question may arise, particularly in case of mud, if radioactivity, sulphuric content, and other mineral salts primarily exert and activating effect or they simply increase the effect, their role being therefore to be considered as an additional factor.

According to our knowledge up to date, in the mud at Hévíz the effective agents are contained in a higher concentration than in the thermal water. At any rate, if we can analyse some of the curative mechanisms of muds, the result will supposedly be valid for the thermal water, too, that is in a permanent contact and interaction with the mud. The biological background of mud formation is already clarified by ZSIRAI and STECKER (1970).

After spreading and success of the molecular biology, however, attention follows more and more the organic macromolecules that are much larger than the inorganic molecules. It is highly probable that the intensive healing effects of the mud at Hévíz are provided for, as well, by the macromolecular organic matters resp. the effects of those activating and regulating solubility, transport of electrons, permeability, and mobility, together with the inorganic compounds.

The aim of our present experiment is to investigate some of the details of the elementary structure of the peat-bug muds at Hévíz, and its free amino acids from among its macromolecular, resp. organic matters, as well as its soluble total protein content.

From the data of germinating seeds in the mud extracts, we are striving to clarify if the medicinal mud has some biological activity. Whether or not it stimulates growing by elongation in the oat-coleoptile test?

We are studying the aerobic and anaerobic bacteria of muds. We are examining if the mud has a bacteriostatic effect.

We are trying experiments to discover the protein-binding capacity of the medicinal mud. We are investigating the microscopic solid frame, resp. the inorganic and organic structure of the mud.

Materials and Methods

The sampling site of the medicinal muds at Hévíz is: 1 = the mud produced industrially from the drainage channel 200 m from the lake, dried and pulverized. This powder is used for treatments, after due dilution and heating. 2 = the natural, ,"wet mud", taken from below water, similarly from the drainage channel, at the same place as before. 3 = wet mud already used for packing treatment. 4 = the natural, wet mud taken from the Lake at Hévíz, north from the spring.

The analyses were carried out from finely pulverized muds dried at 70 °C till constant weight. The organic matter was weighed after being heated (at 500 °C) in oven for five hours. The carbon content was counted in the organic matter, according to FEKETE *et al.* (1967).

For total nitrogen it was lysed according to Kjeldahl. Then, applying protective colloid, we measured the colours of Nessler's reaction by means of a spectrophotometer.

At determining the soluble total protein, it was homogenized with tris ÷ HCl buffer — at 7.5 pH value — and cleared with repeated centrifugation. At the turbidimetric determination of proteins (COLOWICK and KAPLAN, 1957), the data obtained with potassium ferricyanide, TCA and sulphosalicylic acid were averaged. The soluble proteins were measured according to LOWRY *et al.* (1951), as well, with Folin's phenolic reagent. The results were reduced on the basis of the dry-matter content of the wet muds.

The amino-acid content turned out to be extremely small, the extraction was, therefore, carried out from 3.0 g dry matter of muds. Extraction took place with 20 per cent ethanol. We have applied development retarded by coolling and a solvent: butyl alcohol — acetic acid — water (3:1:1), working with ninhydric development and copper-nitric fixation. Identification and quantitative determination were carried out by the universal standard method (PÁLFI *et al.* 1972), on the basis of cadmium-ninhydric colour reaction, by means of a spectrophotometer. At making the thin layer, cellulose powder MN 300 was used.

The mud extracts were examined by the co-work of R. NEHÉZ, with an automatic amino-acid analyser of type "Biocal BC 200", as well, but all the free amino acids, being present but in a small quantity, became disadsorbed. The demonstration is therefore negative. The results of the mud proteins hydrolysed with 6 N hydrochloric acid, obtained by means of an automatic analyser, have aggreed with our total protein data, the details are therefore not published here.

At the biological activity experiments 10 g dry pulverised mud was weighed in, homogenized with quartz sand, in a braying mortar with 30 ml distilled water, for half an hour, and cleared with repeated centrifugation. In the course of germinating the seeds, the filter paper of the Petri dish was moistened with extracts obtained in this way. Extracts like these were used in case of investigating the Avena-coleoptile elongation- and bactericidal effects

At the bacterial investigations, completed solid meat-food agar was used, and every method was carried out according to FERENCZY and ZSOLT (1971). The test-bacteria were obtained from them, too.

Our analyses were performed in 3 to 5 repetitions and the average results of these are published, If the data of repetitions had differred more than ± 5 per cent from the average result (dispersion). the whole analysis was repeated.

Experimental results and their evaluation

The medicinal mud of the peat-bog at Hévíz is produced from the drainage channel of the thermal water rushing up into the lake (200 m from the lake). The soil of the channel and the environment is 6 to 7 m thick peat, getting mud from the lake, too, streaming together with the thermal water. Some data on the composition of the muds in the lake and the drainage channel are shown in Table 1.

It is to be seen from Table 1 that the organic-matter amount of the peat-bog muds is not large and that the organic-matter content of the mud in the lake — as compared to the mud of the drainage channel — is approximately 20 per cent smaller.

The lowest value in total nitrogen, too, is given by the mud of the lake. It may be established, anyway, that all the medicinal muds of the lake at Hévíz are generally very poor in nitrogen. The strong affinity (binding capacity) of the "thermal muds" at Hévíz for the compounds of nitrogen content may be an extremely considerable factor, reflected by the high C:N ratio of Table 1, as well. In case of a C:N ratio as high as this, the nitrogen of the medium cannot be taken up even by bacteria, that is to say, it is quite immobile (FEKETE *et al.*, 1967).

The total protein content of the thermal muds at Hévíz is quite small, as well. An exception is alone the mud already used for packing treatment, containing seven times more protein than the starting matter, *i.e.* the pulverized medicinal mud. These proteins are eluated by the mud in the cousre of the treatment from the surface tissues of the man. The nitrogen deficincy and affinity of the peat-bog muds at Hévíz is, therefore, very high. The protein eluation by the mud from the human organism may be regarded as one of the causes of the curative effect.

It turned out at the exploratory streaks of paper chromatography that free amino acid, as well, is only contained in traces by the muds at Hévíz. We have started, there-

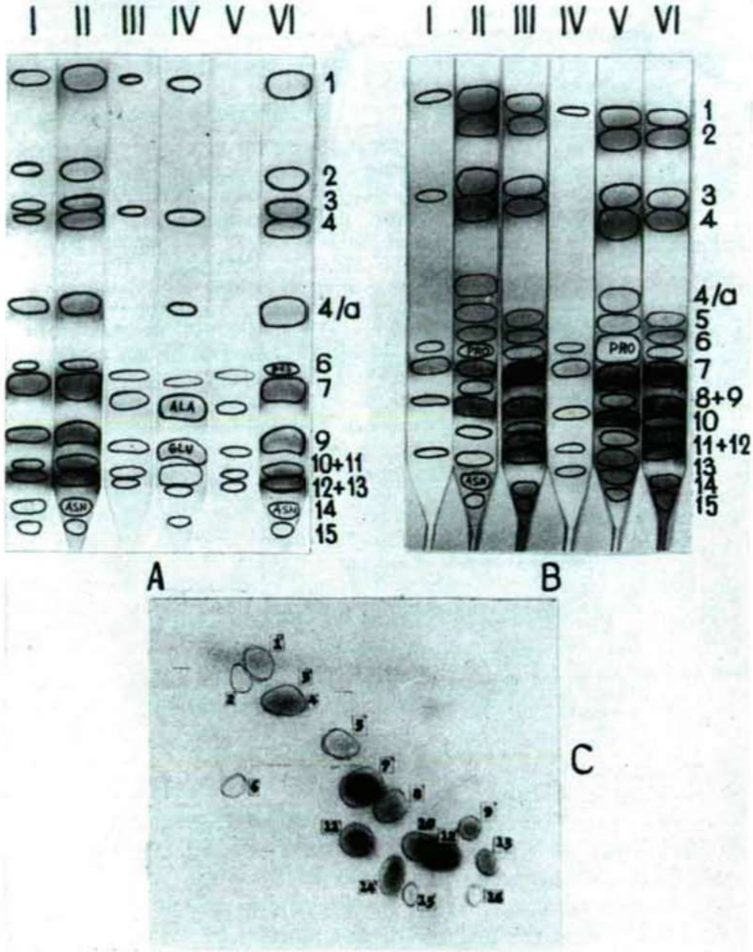


Fig. 1A Free amino acids of peat-bog muds originating from the lake at Hévíz and the drainage channel.

I, II, and VI: Comparative amino-acid standards.

III: Amino acids of the pulverized mud, industrially produced from the drainage channel and dried.

IV: Natural, wet mud, produced from the drainage channel.

V: Mud already used for medical (pack) treatment, evacuated to the lake for regeneration.

1: Leu ÷ Ile;	6: Pro;	12: Ser;
2: Phe ÷ Trp;	7: Ala;	13: Asp;
3: Val;	8: Thr;	14: Asn ÷ His;
4: Met;	9: Glu;	15: Lys;
4/a: γ—Amb;	10: Gly;	16: Cys (I ÷ II);
5: Tyr;	11: Gln;	

Fig. 1B Free amino acids selected at the surface of the human dermal tissue and the "amino acid composition of vegetable type". Numeration is the same as in figure 1.

I: Spinach leaf, fresh, well-watered.

II: Water-deficient spinach-leaf.

III: Human dermal tissue. Sampling took place by washing the surface.

IV: Savoy leaf, fresh, well-watered.

V: Savoy leaf, strongly water-deficient.

VI: Washed amino acids of human dermal tissue.

Fig. 1C Composition of amino acids washed from the 2 sq.cm surface of a natural, living, human dermal tissue.

Two-dimension thin-layer chromatogram. Numeration is the same as in figure 1.

fore, from dry matter of much larger quantity than it is usual in case of vegetable samples. As the mud, in the cousre of packing, touching human dermal tissues, is getting into interrelationship with them, we have washed samples (with water and brush) from the surface of the living, human dermal tissues, as well, that is from the epidermis, for the purpose of amino-acid analysis. There were elaborated vegetable samples, as well, for being compared. (Fig. 1, A, B, C.)

In chromatogram "A" of Figure 1, there are applied three kinds of standard amounts (stripes I, II, and VI). But the mud-extracts (stripes III, IV, and V) did not achieve the lowest-standard total amino-acid amount (15 μg), either.

A comparatively higher amino-acid concentration was shown by the wet mud of the drainage channel of the lake at Hévíz what is due to the meeting of three conditions: 1 = the human skin surface; 2 = the bath-water and mud respectively; 3 = very much urea.

The water is quasi cleansed by the mud disturbed by bathers. That is to say, the mud is binding organic compounds and microorganisms at its active surfaces, deposing in the drainage channel in that way.

From the mud the following amino acids could be demonstrated: leucine ÷ isoleucine, valine, methionine, γ-aminobutyric acid, proline, alanine, glutamic acid (the most, together with alanine), glycine, serine, aspar-tic acid, and lysine. The amino-acid spectrum of the thermal mud at Hévíz (Fig. 1/A, III, IV, V) turned out, by the help of stripes I and IV in Fig. 1/B, to be similar to that of the plants grown under optimum conditions, its composition (origin) being therefore "of the same type as that of the higher plants". However, the qualitative composition is only similar as in case of muds we departed from thirty times more substance than in case of plants. The leaf of plants is rich in free amino acids at which, owing to water deficiency, the protein-synthesis is stagnant. (Fig. 1/B, stripes II and V.) At the same time, the amino-acid synthesis — as a defensive reaction — becomes stronger (PÁLFI, 1968; PÁLFI *et al.*, 1973, 1974). It is to be seen on stripes III and VI of Fig. 1/B that the amino acid of the most quantity was obtained by washing from the living, human dermal surface and this composition is quite complete for the protein syntheses, as well. According to our measurements, from the surface of the living human dermal tissue of a man, on the occasion of a single bath taken, on the average more than 2.0 grammes free amino acid is dissolved and lost in five minutes. In an hour, however, this loss may amount even to 6 to 10 grammes (according to the level of protein nourishment). On the other hand, the thermal water becomes richer in compounds of nitrogen content (amino acid and urea). A change in the composition of organic matters and microflora — that are "stable" for a long time and provide for the curative effect of the thermal water — can just be induced by becoming richer in nitrogen and shifting (degrading) the C:N rate in that way. It is therefore unadvisable to augment the number fo bathers in the lake considerably (by normal bathing tourists). A "free open-air bath" fo considerable surface would be worth while to be developed from the drainage channel of the thermal water.

According to the spectrophotometric measurements of the cadmium-ninhydrin colour-reaction, most amino acid is found in the wet mud got from the drainage channel (86 mg/kg dissolved from the bathers). The amino-acid content of the mud dried and pulverized industirally is lower (52 mg/kg) as some of the amino acids are bound by the aerobic bacteria. Lowest is that of the mud used for packing (strongly aerated) and that of the anaerobic, wet mud of the lake at Hévíz (equally 31 mg/kg).

The free amino acids of the organic matter of some soils were investigated by FILEP and TATÁR (1974). The results obtained by them are fairly agreeing with our amino-acid amounts demonstrated from the peat-bog muds at Hévíz.

In Fig. 1/C, the spectrum of some amino acid washed from the epidermis of a 2 sq.cm living, human dermal tissue is to be seen, at a two-dimensional layer-chromatogram. It can be established from the figure that at the epidermis of a well-nourished man a very considerable quantity of amino acid is secreted.

In the course of the germination of seeds a very intensive metabolism and rapid growing are taking place. The effects of the environmental factors and among them those of the biologically active substances are therefore sensitively indicated by the germinating plants.

In Fig. 2/A and B, white mustard, resp. lettuce plants are to be seen after germinating for four days. The five plants on the left have germinated in the medium of mud extract, and the five plants on the right did it in running tap water (controls).

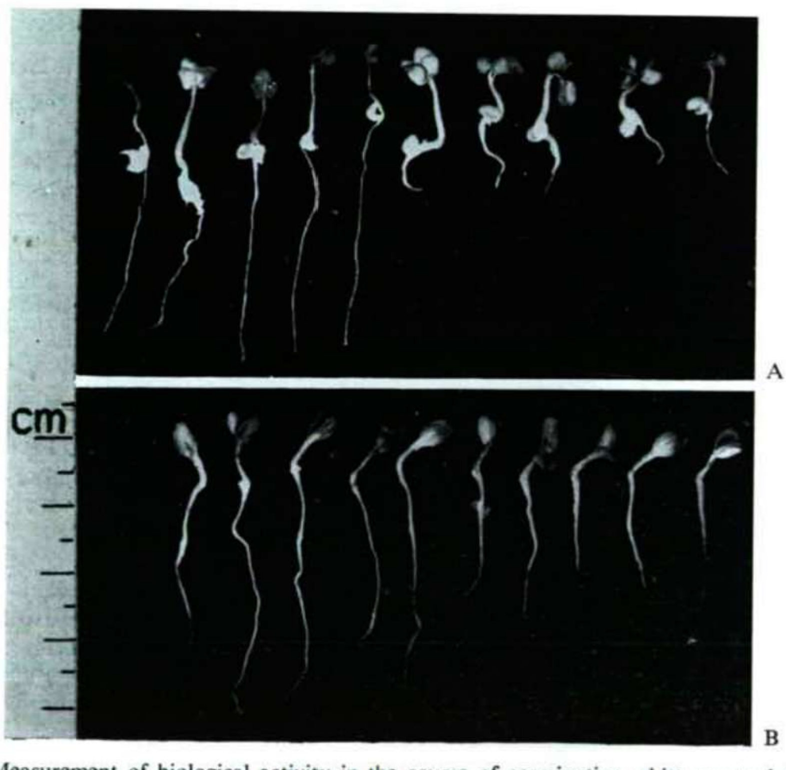


Fig. 2A Measurement of biological activity in the course of germinating white mustard (Sinapis alba L.). The five Plants on the right were germinated in tap water, and the five plants on the left in a medium of aqueous mud extract. In the elongating growth of roots, 400—500 per cent stimulations was achieved with mud extract.

Fig. 2B Growth-stimulating effect of the extract of peat-bog mud on the germination of lettuce (Lactuca sativa L.) seeds. The five plants on the left were germinated in the extract of mud, resulting in 100 per cent root-growth stimulation. The five plants on the right are wet controls.

From Fig. 2 we can establish that in the extract of the peat-bog mud a 100 to 500 per cent promoted root growth was materialized! This biological activity is the second cause inducing the curative effect. And the metabolism-stimulating matter may have been a humic-acid derivative as in the organic matter of the peat-bog mud at Hévíz about 10 to 15 per cent total humus was contained (FEKETE *et al.*, 1967).

ZIECHMANN and PRZEMETCK (1964) were investigating on root meristem the effect exerted by natural and artificial humic matters on root-growing and phosphatase activity. The authors demonstrated a considerable stimulation of growing.

SÍPOS *et al.* (1974) investigated the flat bog-peats at Keszthely and established that in case of a pH value lower than 3.0 humic-acid aggregates are present. At a higher pH value these aggregates decompose. It follows from that that the extract of the peat-bog mud at Hévíz that is showing 6.8 pH is mainly the aqueous solution of monodisperses of smaller molecular weight.

According to BUTLER and LADD (1969, 1971), the intensity of the proteolytic enzyme-acitivity is considerably influenced by humic acids. And in this respect the molecular weight of the humic and fulvic acids manifest itself as a regulating factor.

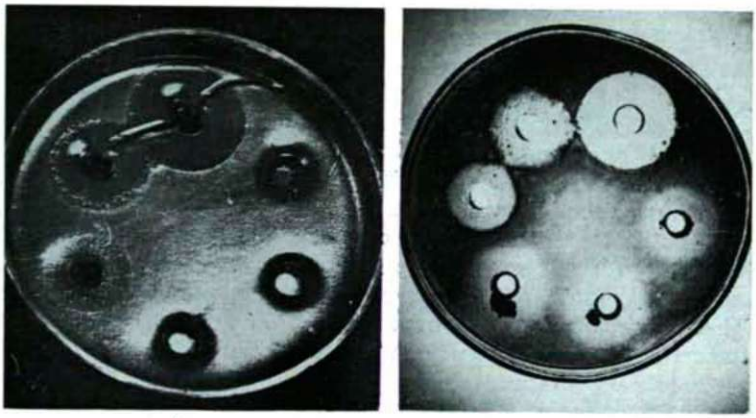
We have investigated the microflora of the peat-bog mud at Hévíz, as well. There were produced pure aerobic cultures by means of surface-streaking isolation.

The aerobic bacterial flora turned out soon to be extremely poor in species. Colonies were only formed by *Bacillus megc. rium, Bac. cereus* var. *mycoides, Bac. subtilis,* and *Sarcina lutea*. All these are saprophytic, proteolytic, typical aqueous bacteria. The colonies of an unknown bacterium from among the species multiplying in the presence of air were isolated, too. This bacterium of giant size, whose length attains even 20 to 24 μ, is Gram-positive, sporular, and capsulated (Fig. 3). It proved to be an involutional, degraded species. A total aerobic bacterial germ-counting was

Fig. 3. Microscopic picture of the unknown aerobic bacterium. Gram-positive staining. The division in tens of the ocular-micrometer corresponds to 8 μ. They are large-sized, spore-bearing, capsular bacteria, without independent movement. Their colony is butteryellow, bright.

also carried out, demonstrating 22 thousand, reckoned over into 1 gramme dry mud. This number is extremely low and may have been a consequence of nitrogen deficiency in the mud.

It was examined, similarly in aerobic cultures, if the extract of the peat-bog mud at Hévíz had a bacteriostatic effect. As test-bacteria were used *Staphylococcus aureus* and *Escherichia coli*. As compared to streptomycin (25, 50 and 100 mg/l.), the mud extract was during two days only weakly bacteriostatic. But at cultures of four days the mud turned out to have a quite considerable bacteriostatic effect (Fig. 4, "A" and "B"). That may be a second main cause in inducing the curative effect of the mud.



A B

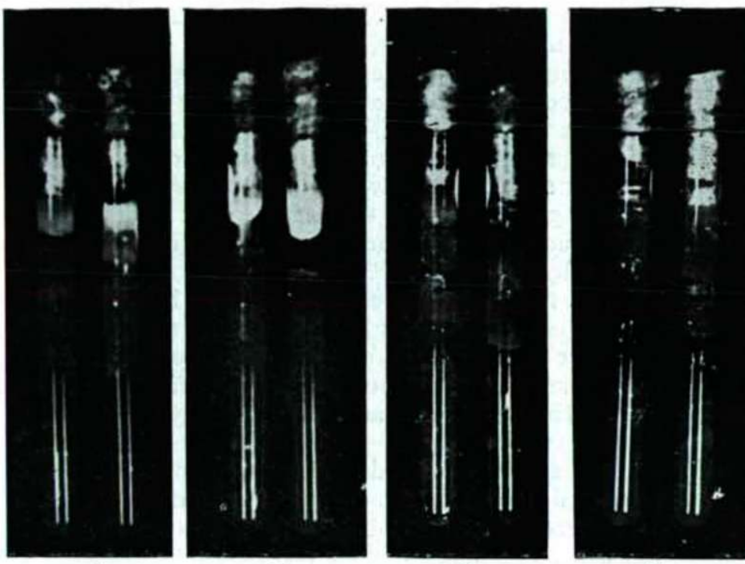
Fig. 4A Antibacterial effect of the mud at Hévíz. The culture medium was uniformly covered by the colonies of Staphyloccocus aureus after inoculation with surface streaking. The there left upper cavities were filled in with streptomycin solution; 25, 50 and 100 mg/l (control), and the three right lower ones with mud exrtracs, Photographing in side-light. The mud-extracts, diffused out of the cavities, inhibited the development of bacterial colonies at a considerable surface. 4-day old cultur.

Fig. 4B Bacteriostatic effect of the peat-bog mud at Hévíz. The 4-day od Escherichia coli culture was illuminated from below.

At multiplying the anaerobic bacteria, three were applied deep inoculations into the solidifying culture media and the withdrawal of oxygen was provided for with some strongly alkaline pyrogallol (in a closed space).

It is shown by figures 5/A and B that a lower number of anaerobic colonies are formed by the industrially produced, pulverized mud and the mud already used for packing, and that their metabolism is lower, as well, as they don't develop any gas. These two kinds of mud have communicated and mixed with air and that is not favourable for the multiplication of anaerobic bacteria. The anaerobic inoculations of mud samples got from below the thermal water (Figs. 5/C and D) formed more colonies than the others before, with an extremely strong gas development. The culture medium was disrupted, squashed by the gases (mainly CO_2) produced in the course of the bacterial metabolism. There could be observed a similarly strong gas generation after moving or treading the mud in the lake. In the anaerobic muds (under water) hydrogen sulphide occurs but in traces; even the highly responsive silversulphide test is not functioning with its quantity. Under aerated conditions that can, of course, be modified (in the course of mud production and packing).

In case of anaerobic bacteria more species can have a part than in case of aerobic ones. The leading figure was genus Clostridium.



A B C D

Fig. 5. Virulent anaerobic bacteria in the medicinal mud at Hévíz, in case of excluding air. Non-pure cultures. Test-tube "A" was inoculated, with industrially produced pulverized mud, test-tube "B" with mud already used for packing. These two samples were already mixed with air for a long time (The grey colonies are to be seen inside the agar).
"C" and "D" are samples obtained from below the thermal water of the lake and drainage channel respectively, that is under anaerobic conditions. The culture medium has been inflated, smashed by the gas generated during the intensive metabolism.

A first-rate restricting factor of a higher-class multiplication of the anaerobic microorganisms are the strong nitrogen deficiency of the mud and consequently the extremely high C:N rate (Table 1). The considerable stability and evaluative curative effect of the composition of the chemicals and among these mainly the organic matters of the mud at Hévíz are provided for just by this high C:N ratio, as well as by the few fluctuations in the temperature of the thermal water and in the quantity of its dissolved substances. An increase in the nitrogen level of the thermal water and mud respectively can be released, for instance, by a considerable increase in the number of bathers in the lake that considerably increases the concentartion of the free amino acids and peptides solved from the dermal surfaces, as well as that of the urea and organic nitrogen compounds and by evacuating the muds used for packing into the lake. Then the C:N rate changes, it may decrease strongly.

The counting method of the anaerobic bacteria at Hévíz would demonstrate not the real vital functions but only the presence of spores in a large quantity. We did not deal therefore with that.

We have also examined if the extract of the peat-bog mud at Hévíz is containing some matters stimulating growing by elongation, like indole-acetic acid (IAA). It is known that IAA originates from a protein forming amino acid, tryptophan. This amino acid, however, could not be demonstrated from the mud. The length of oat coleoptiles germinated without light (etiolated) and decapitated will be extended by IAA 30 to 40 per cent, if floated in its solution for 24 hours. And a similar effect may be elicited by some steroid hormones, too.

The concentrated solution of the mud extract was investigated pure and combined with IAA, with 10^{-5} Mol solution of IAA and a control with distilled water, and later the effect of its avriations in 2-, 4-, 8-, 10-, and 20-, 50-, 100-, 200-, and 1000-fold dilutions. We have established that the mud-extracts do not show (even in traces) any hormonal activity for stimulating growing with elongation.

It is proved by the negative results of the oat coleoptile test that the stimulation of root-growth by the mud extracts in the course of the germination of seeds was not founded on growing by elongation.

We were also striving to clarify if there is shown such a high affinity and binding capacity in respect of certain compounds by the peat-bog mud at Hévíz, as it was already reported on in case of the peat at Keszthely, Small-Balaton, and at other sites. (FEKETE, 1952; BELÁK *et al.*, 1970; LAKATOS *et al.*, 1974; SÍPOS *et al.*, 1974; SZALAY *et al.*, 1974; and 1975). The authors are attributing the high-level adsorptive capacity of peat-bogs to the humic acids.

In our experiment, we have measured the known quantitites of "bovine serum albumin" and admnistered these to the muds, incubating them at 40 °C for twenty minutes. Then we measured back the soluble total protein quantities from the mud.

It can be estabilished fromTable 2 that the protein-binding capacity of the peat-bog mud originating from the lake at Hévíz is quite great. There could demonstrated only 1/10 to 1/4 part of the total protein quantity admixed to the mud artificially. The larger part of the protein quantity admixed to the mud was strongly bound by the humic acids and the solid frame of mud. Our results are confirmed by the work of others, as well. KLÖCKING (1967) was also studying the protein-binding capacity of humic acids and demonstrated *in vitro* an extremely active complex-forming and adsorptive effect.

SOGINOV (1961) revealed some peculiarities of the "humoprotein-complexes", too. The new method of obtaining water-soluble humic acids from the soil was published by MÜCKE and KLÖCKING (1966). There were also elaborated some methods for isolating the protein compounds of humic acids originating from the soils (SIMONART *et al.*, 1967).

From Table 2 we may come to the conclusion, as well, that the 7.0 mg protein demonstrated by LOWRY *et al.* (1952) with Folin's phenolic reagent may correspond to 40 mg, in case of the mud used for packing (Table 1). In course of packing, a considerable number of protein-like compounds, streaming from the human organism, are bound.

The curative effect of mud pack may take place according to the following mechanism: as a result of the considerable local heat effect of the hot mud pack the pores of skin quite open. The blood- and oxygen-circulation, as well as the nutrient-metabolism of the treated part of tissue, increase many times (at the expense of other, non-activated body parts).

The water-soluble and salt-soluble humic acids, available in great superabundance (in perspiration a large quantity of sodium salt is secreted) as exchange-electrolytes are passed down by the mud through the dermal tissue towards the interior. The metabolism continues to be accelerated by the humic acids and derivatives having got into the surface tissues as biologically active compounds that have a considerable electron-surplus.

According to BELÁK *et al.* (1969, 1970), LAKATOS *et al.* (1974), and SZALAY *et al.* (1974, 1975), as well, peats have reducing particularity that is to say, they have a part as electron-donors.

The humic acids streaming into the tissues, and other concomitant inorganic and organic compounds, may increase (disintegrate) the degree of dispersity of "foreign proteins" that is of antigen-antibody aggregates deposited in the painful joints and then, as protecting colloids they promote solution and hydrophilism in colloidal way. Then passing some of their electron-surplus to the protein aggregates, they initiate their mobilization. Consequently, the own "reacted" electron transport of the so far chemically indifferent protein aggregates of damaging effect can start again. The streaming of proteins already in motion and having an electron-structure towards the dermal tissues may also be promoted by that the damaging protein-like compounds — that with the perspiration components are approaching the surfaces — are almost "attracted" by the nitrogen-affinity of the mud and bind those after forming biocomplexes with them. These suppositions, founded on our results, are supported by Breznay's (1970) experiences and publication, as well. According to SZALAY *et al.* (1974), the sorptive properties of the peat sample may be compared in qualitative way to a "cation exchanging synthetic resin" that contains carboxylic groups on a poly-aromatic frame, and is available at shops.

The healing of the wounds, ulcers and scleroses of the chronic inflammatory tissue regions of famela patients are just promoted by aqueous extracts, press juices, and squeezes of muds. In the part-mechanisms of healing the bacteriostatic effects proved by us and the physiologically active matters of the muds of Hévíz, resp the electrostatic humic acids and their derivatives may be responsible.

The beginning, development, and composition of the peat-bog mud in the medicinal lake and its drainage channel considerably differs from the properties of the peats in this region in the country (Table 1). The standing communication of mud with the "thermal water" (heat transmission), their close physical, chemical (high C:N ratio, resp. the immobile nitrogen content), and biological interactions, as well as the anaerobic conditions are to be considered as a difference-inducing considerable conditional impact. In the latter species a characteristic and stable microflora combination is ensured. The continuous moving of mud by the flowing thermal water, its stirring by bathers, as well as the rather strong squirt of the thermal spring, throwing up the muds that obstruct its mouth and spreading them again over the whole lake: all these proved to be essential.

We should like to clarify, as well, what kind of solid structure these mud components of a strong adsorptive capacity in fact have. For this purpose, the pulverized mud industrially produced from the drainage channel at Hévíz and that of brought to the surface of the lake was examined with microscope. We are presenting here some of the many hundred microscopical photographs (Figs. 6/A, B, C, D).

In the pictures of figure 6, the remains of vegetable cell frameworks, resp. parenchymal cells (A and C) as fenestrated reticula and spongy-porous matters of large surface are to be seen. In the upper part of figure 6/B long cells of thickened

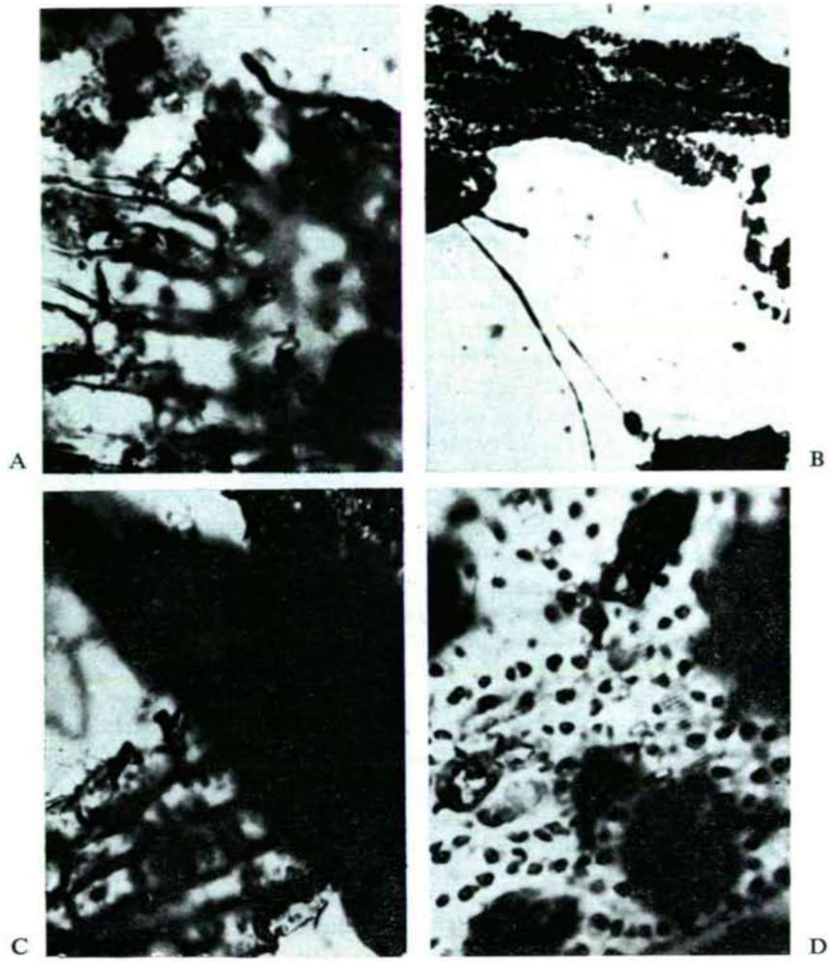


Fig. 6A The plant-originated solid structure of the mud at Hévíz. Parenchymatous tissue. x1500. Dark organic compounds, humic acids are bound by the frame of cellular pattern at its surface.

Fig. 6B Microscopic remains of the solid structure of mud. At the surface a lengthwise running conductive element of thickened wall, some filamentous green alga, as well as an inorganic amorphous detail, too, are to be seen. x1500.

Fig. 6C In the middle there is some clay-mineral, at the surface with dark humic-acid compounds bound. Below: parenchymatous cells are as a cellular net-pattern. x1500.

Fig. 6D Epidermal-tissue cuticle of the leaf of a higher plant with the characteristic cones. To its surface, inorganic, amorphous granules are adhering. x1500.

wall, *i.e.* details of conductive tissues appear, and in the lower part filamentous algae. (Fig. 6/D)=Cuticle of leaf with pins. In each of the pictures in figure 6, mineral dark, amorphous details are also to be found. It may be established that these solid structures themselves can function as filtering networks if they are stirred up in the covering water. 2/3 part of the volume of mud is formed by some structures of vegetable origin like these. The specific weight of this moving microdetritus is only a few decimals larger than that of water, and the specific weight of the mineral substances is 2 to 3. These fragments of cells and tissue-networks, too, have a part in developing the strong adsorption opposite to foreign matters. In addition we may find plenty of filamentous green algae and Diatoma, as well as the pollens of mono- and dicotyledons in the mud. The inorganic constituent of the peat-bog mud is either an amorphous or an in its majority crystalline granule. The inorganic and organic structure-components appear "pure" only rarely. At their surfaces they often bind coloured substances that are mostly not or hardly water-soluble, bacterial metabolic products, and mainly humic acids. The colour of structures is therefore dark.

The extraordinary efficaciousness of the peat-bog mud at Hévíz, able to adsorb, bind the compounds of organic resp. introgen content, is ensured apart from its peculiar chemical composition (its humic acids, resp. active substances) by this net-like and spongy microdetritus-structure of vegetable-tissue origin.

Finally, it is to be mentioned that the thermal water is continuously mixed with the mud dissolving the substances of that. The thermal water and the curative active agents of the peat-bog mud are, therefore, in a standing interaction with one another. It follows from that that during the balneoligical treatment, as well, there are mostly the same matters that are exerting their influence — possibly in another concentration than in the time of mud pack. The differences between the agent-quantities are anyway balanced by that the whole body surface is in junction with the medicinal water (except for the head) and the duration of impact on it is much longer, too, than in case of mud pack.

The daily alternation of balneological and mud treatments is presumably efficacious for that reason.

Table 1

Peat-bog mud samples got from below the lake at Hévíz (Medicinal muds) Flat peak-bog pH=6,8	Organic matter	Carbon	Total nitrogen	C:N rate	Soluble total protein mg/g dry matter
	In the percentage of the dry matter				
The industrially produced, dried, and pulverized mud from the drainage channel	51,3	24,1	0,72	33,4	1,1
Wet mud got from the drainage channel (from the site of production)	51,5	24,2	0,76	31,8	1,5
Wet mud, already used for pack treatment	52,1	24,5	0,92	26,6	7,0
Natural, wet mud got from the lake at Hévíz north from the spring	42,6	20,0	0,70	28,5	1,0

Table 2

Protein quantity portioned to 1 g of mud (dry matter) mg	Demonstrated by nephelometry	Measured by Folin's phenolic reagent
	soluble total protein	
2	1,1	1,2
5	1,2	1,3
10	1,8	2,0
20	4,2	4,5
40	5,6	7,0
80	18,3	20,1

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Table 1. Organic-matter, carbon, and total nitrogen content of the peat-bog mud in the lake at Hévíz and its drainage channel, as well as C:N ratio and total protein concentration.

Table 2. Post-incubation extraction of the soluble protein (bovine serum-albumin) administered to the medicinal mud at Hévíz, as well as its quantitative demonstration with nephelometry and Folin's phenol reagent.

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